Determination of the *dha* regulon of *C. werkmanii* and characterization of Δ*dhaD*

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Citrobacter sp. is a poorly investigated micro-organism with a lot of interesting properties in different areas. One property is that they can precipitate heavy metals like sulphate and copper (Qiu et al. 2009). This phenomenon may be useful in the bioremediation of acid mine drainage. Another quality is that the bacteria can grow on glycerol as the sole carbon source (Bouvet et al. 1995). The genes responsible for this characteristic are structured in the *dha* regulon. This regulon is already characterized for *Citrobacter freundii* (Daniel et al. 1998). However, when a PCR was performed on the genome of *C. werkmanii* with primers for different parts of the regulon based on the *dha* regulon of *C. freundii*, only one product was positive. So the *dha* regulon of *C. werkmanii* was explored through genome walking. This resulted in a regulon with a similar organization as the regulon of *C. freundii*. Moreover, the deduced protein sequence showed 98,8% identity compared to the regulon of *C. freundii*, 70,2% identity with *C. koseri ATCC BAA-895* and 96,8% with *C. youngae ATCC 29220*. In a second phase of this study, the *dhaD* gene coding for the glycerol dehydrogenase was knocked out using the one-step inactivation method (Datsenko and Wanner 2000). This mutant was characterized by sequence analysis and an enzyme assay. To our knowledge, this is the first knockout mutant made in *Citrobacter* sp.

Keywords :
Citrobacter werkmanii, *dha* regulon, knockout mutant, glycerol dehydrogenase

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